position 171) is the *HBM* gene and its corresponding protein. *See* "Brief Description of the Figures" for Figures 6A-6E.

The high bone mass ("HBM") phenotype was identified in a Midwest family. Johnson et al., 1997 "Linkage of a Gene Causing High Bone Mass to Human Chromosome 11 (11q12-13)" *Am. J. Hum. Genet.* 60: 1326-1332 (attached). Specifically, a teenage girl who presented at a hospital after a car accident was X-rayed and found to have extremely dense bones. *Id.*, at 1328. Her family was studied and the phenotype expressed in this family was found to be linked to a specific, single genetic locus. The linkage study that identified the locus is generally described in Johnson et al. (1997) and is also present in the Specification. The HBM variant of the *Zmax1* gene responsible for this higher than normal bone mineral density was isolated and disclosed in related patent applications filed April 5, 2000 (U.S. Serial Nos. 09/544,398, now U.S. Patent No. 6,770,461 and 09/543,771 now U.S. Patent No. 6,780,609) and January 13, 1999 (U.S. Serial No. 09/229,319, now abandoned); the instant application claims priority to these U.S. patents and application. The LRP5 gene and its polymorphic variant are also described in a paper by the inventors. *See* Little et al., 2002 "A Mutation in the LDL Receptor-Related Protein 5 Gene Results in the Autosomal Dominant High-Bone-Mass Trait," *Am. J. Hum. Genet.* 70: 11-19 (attached). The HBM phenotype (*i.e.*, higher bone mass or density) was shown to track 100% with the gene containing the HBM polymorphism. *See, e.g.,* Specification at p. 74, lines 9-10; Little et al., at 11. Thus, a monogenic cause for the high bone mass phenotype was identified.

An epidemiological correlation between osteoporosis and cardiovascular disease had been long observed. Parhami et al., 2001 *J. Bone & Min. Res.* 16: 182-188, 182.[1] Low bone mineral density ("BMD") is associated closely with cardiovascular disease mortality, cardiovascular calcification, atherosclerosis, and high lipid levels. *Id.* One of the papers referenced by Parhami *et al.* regarding the BMD/high lipid level correlation dates back to 1972 (Pinals et al., 1972, "Type-IV hyperlipoproteinemia and transient osteoporosis," *Lancet* 2: 929; attached). Risk for atherosclerosis increases with increasing concentrations of low density lipoprotein ("LDL") cholesterol, whereas risk is inversely proportional to the levels of high density lipoprotein ("HDL") cholesterol. Krieger, 1998 *Proc. Natl. Acad. Sci. USA* 95: 4077-4080, 4077 (attached). Hypercholesteremia is a disease characterized by elevated plasma LDL cholesterol and premature atherosclerosis and heart attacks. *Id.* Plasma levels

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[1] Submitted as Exhibit B of the Appeal Brief dated July 2, 2004.

of HDL cholesterol strongly inversely associate with atherosclerotic cardiovascular disease. Jin et al., 2002 *Trends in Endocrinology & Metabol.* 13: 174-178, 174.[2]

After identification of the high bone density in individuals expressing the *HBM* variant, Applicants conducted further studies to determine whether the HBM gene also caused lipid levels to be altered in the members of the kindred expressing the *HBM* gene and associated high bone density phenotype. Example 3 of the Specification tested this hypothesis to see whether the *HBM* gene was involved in lipid regulation. Specification at pages 125-128. The Specification states:

> [B]iochemical tests were performed to measure serum level of various lipid containing molecules or precursors in affected and unaffected HBM family members to test whether the HBM mutation in the Zmax1 gene effects lipid metabolism. . . . The results obtained were statistically significant: (1) Triglyceride levels were generally lower in affected individuals than in unaffected individuals, and (2) very low density lipoprotein (VLDL) levels were generally lower in affected individuals than in unaffected individuals. Additionally, the following comparisons approached statistical significance (p=0.06): (1) high density lipoprotein (HDL) levels were higher in affected males than in unaffected males, and (2) the ratio of low density lipoprotein (LDL) to high density lipoprotein (HDL) was generally higher in affected males than in unaffected males.

Id. Based on this human *in vivo* data and in view of the historical background, it would be evident to one of ordinary skill in the art to use the *Zmax1* and *HBM* genes and associated proteins in methods to identify agents that modulate the lipid level of a host to whom this agent is administered given the teachings of the Specification.

Since the findings set forth in Applicants' specification, additional data has been published by both named inventors as well as by other non-biased third parties that strongly supports Applicants' findings. For example, Fujino et al. describe a knock out of *Lrp5* in mice. Fujino et al., 2003, "Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion," *Proc. Natl. Acad. Sci. USA,* 229-234.[3] In the paper, the authors "describe a function of LRP5 in the metabolism of cholesterol and glucose." *Id.*, at 229. The authors' findings showed that *Lrp5* null animals were hypercholesterolemic (*i.e.,* high levels of plasma cholesterol), had increased levels of very low density lipoprotein (VLDL) cholesterol, and exhibited delayed

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2 Submitted as Exhibit E of the Appeal Brief dated July 2, 2004.
3 Submitted as Exhibit C of the Appeal Brief dated July 2, 2004.

clearance of chylomicron remnants. The data presented by Fujino et al. indicated ***that Zmax1 is a multifunctional receptor involved in multiple pathways, including bone development, cholesterol metabolism,*** and the modulation of glucose-induced insulin secretion. *Id.* These data confirm Applicants' data set forth in Example 3 where it was shown that the *HBM* allele of *Zmax1* results in the exact opposite effect on lipid levels to those reported by Fujino et al. Together, these data show from both human and mouse genetic models, that alleles of *Zmax1* are directly responsible for a continuum of lipid level phenotypes.

In another article by the same group, the authors investigated the role of LRP5 in lipid metabolism. Magoori et al., 2003, "Severe Hypercholesterolemia, Impaired Fat Tolerance and Advanced Atherosclerosis in Mice Lacking both LDL Receptor-Related Protein 5 (LRP5) and Apolipoprotein E," *J. Biol. Chem.* 278(13): 11331-6.[4] The authors compared plasma lipoprotein levels in *Lrp5* knock out animals, Apolipoprotein E (ApoE) knock out animals, and in animals wherein the genes for both *Lrp5* and *ApoE* were knocked out. The data showed that LRP5 is involved in plasma clearance of intragastrically loaded triglyceride and was involved in the development of atherosclerosis in the knockout mice. The authors concluded from the data obtained that ***LRP5 "mediates both apoE-dependent and apo-E independent catabolism of plasma lipoproteins.***" Magoori et al., at 11331. It should also be noted that mice deficient in LRP5 have also been reported to have low bone mass, low body weight, and abnormal eye vascularization. Kato et al., 2002, "Cbfa1-independent decrease in osteoblast proliferation, osteopenia and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt co-receptor," *J. Cell Biol.* 157: 303-314 (attached); and Fujino et al. (2003).

Demonstrating that a change in phenotype (*i.e.*, lipid metabolism and bone mineralization) is correlated with a change in genotype (*i.e.*, knock out animal models of *Zmax1* or the HBM kindred) is a basic and universally accepted way of demonstrating that a protein gene product, such as Zmax1 and HBM, is directly involved in control of a phenotype. The fundamental understanding of the relationship between genotype and phenotype establishes the genotype as cause and phenotype as effect, because it is known that where phenotype follows genotype no earlier condition is a common cause of the correlated phenomena (*i.e.* the phenotype and genotype). Therefore, the conclusion of causation that underlies the asserted utility of the presently claimed methods is not spurious.

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[4] Reference was submitted in manuscript format as published on the Internet in Exhibit D of the Appeal Brief dated July 2, 2004. The reference is newly submitted as reprint from *Journal of Biological Chemistry*.